Hepatoprotective activity of the crude leaf extract of *Ipomoea batatas Linn.*against acetaminophen and alcohol induced liver damage on Sprague-Dawley rats.

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ABSTRACT

Ipomea batatas with the local name of Kamote, is the one of the most common edible and useful plants in the Philippines. This study investigated the protective effect of the crude leaf extract of *Ipomoea batatas Linn.*against acetaminophen and alcohol induced liver toxicity in rats. The pretreatment was conducted for seven days were done on the three groups, each composed of five rats, and received NSS (3ml), Livatone® (400mg/kg) and flavonoids (400 mg/kg) respectively via oral route using normal saline solution (3ml). The induced hepatotoxicity was done my administering acetaminophen (500mg/kg) dissolved in normal saline solution (2ml) and 18% ethanol (5ml/kg) via oral gavage into the rats. Alanine transferase, aspartate amino transferase and total bilirubin were measured three times after the treatment and feeding process. Based on studies, normal values of ALT, AST and bilirubin should be from 0.00 to <40 U/L normally and an elevated value of any of the said analytes indicates damage on the liver. Analysis of data revealed that there is a significant difference between the effects of the crude extract and the negative control on all test parameters indicating its hepatoprotective potential. Furthermore, it was noted that the crude extract of *Ipomea batatas* at a dose of 400 mg/kg body weight produced the same hepatoprotective effect as Livatone® at the same dose using a 95% confidence interval. Further studies on the specific hepatoprotective effects of the primary and secondary metabolites of the crude extract is recommended.

Keywords: *Ipomoea batatas Linn*, flavonoids, hepatoprotective effect

INTRODUCTION

The liver is the largest organ of the human body and also one with the most functions. It is the vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges like xenobiotic, drug viral infection and chronic alcoholism (Chaudari et al., 2009). It is through the liver that blood supply passes through. It both produces and secretes bile, as well as the two blood-clotting factors of prothrombin and fibrinogen. It also produces heparin, which on the other hand,

prevents blood from clotting. Another important function of the liver is to convert sugar into glycerine (Sunilson et al., 2009).

Due to the vital role of the liver in the human body function, its impairment due to diseases is a major concern as it causes morbidity and mortality. Liver diseases are the most serious ailment and are mainly caused by hepatotoxins like consumption of alcohol, high doses of paracetamol, carbon tetrachloride and chemotherapeutic agents that overpower the protective mechanism of the liver and cause hepatic damage (Bhawna et al., 2010; Anandan et al., 2009). Liver disorders ultimately cause death.

Liver hepatotoxicity caused by paracetamol overdose can be fatal. It is now the most cause of the potentially devastating liver failure. Most of such instances are the consequence of ingestion of large paracetamol, often taken at a single time-point with suicidal or parasuicidal intent (Kurtovic et al., 2003). Paracetamol or acetaminophen (APAP) is one of the most commonly used medications worldwide (Kuvandik et al., 2008). Another common cause of hepatotoxicity is the alcohol; it can cause fatty liver, alcoholic hepatitis and potentially, cirrhosis of the liver (Prescot et al., 2000). Alcohol-induced liver injury causes serious medical, financial and social problems. The disease progresses from fatty infiltration and follows a pernicious course of inflammation leading to irreversible liver damage. It is well known that chronic ethanol ingestion produces fatty liver, hepatomegaly, alcoholic hepatitis, fibrosis and cirrhosis (Mandal et al., 2006).

Natural remedies are being resorted to combat the disease. A lot have proven to have therapeutic effects because of their curative properties some which are; phenols, coumarin, carotinoids, xanthenes and flavonoids. Plantcures are proven safe, non-toxic and with no serious side effects (Bhawna et al., 2010).

Ipomea batatas with the local name of Kamote, is the one of the most common edible and useful plants in the Philippines (Garcia, 2010). Several studies were done to different variety of plants regarding with its flavonoid content and its antioxidant properties. And it shows that both green and purple leaves of sweet potato scientifically known as *Ipomea batatas* has high amount of flavonoids (Yan-Hwa et al., 2000).

Flavonoids are one of the most prevalent classes of phenolic compounds widely distributed in edible plants and thus are important constituents of human diet (Ji-Yeon et al., 2002). These are natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds (Nijveldt et al., 2003). They are large compounds occurring ubiquitously in food plants and are natural compounds known to have properties to guard against inflammation, allergy, virus, bacteria and tumor. They are said to have antioxidant, hepatoprotective and pharmacological effects because they are able to inhibit certain enzymes (Yan-Hwa et al., 2000; Molina et al., 2003; Babenko et al., 2008).

There are currently no reports regarding the hepatoprotectivity of the leaves of *Ipomea batatas Linn*. For this reason, this study investigated the hepatoprotective activity of the infusion of the leaves of *Ipomea batatas*. Based on the results, verify the claim of using paracetamol and ethanol induced hepatic injury model in rats.

MATERIALS AND METHODS

Drugs and Chemicals

Paracetamol procured locally was used to induce hepatotoxicity in laboratory animals. Alcohol-caused hepatotoxicity was induced using a locally manufactured beer. Livatone® (Cabot Health, Australia) were used as the control. All chemicals that were used are of analytical grade.

Plant Material

Fresh roots of *Ipomoea batatas Linn.*were planted directly in the soil with standard environment conducive to its growth where there is enough sunlight and consistent spray of water twice a day. The said plant were planted, grown and collected in P. Burgos St. Brgy. 11 Batangas City. After a month, veins and leaves are expected to be fully expanded (Huang et al., 2004). The green leaves were harvested and identified by the Herbarium of the Thomas Aquinas Research Complex of the University of Santo Tomas with authentication number USTH-5541, and used for extraction.

Sample Preparation

The fully expanded green leaves of *Ipomea batatas Linn.* (100 grams) were cleaned, and air dried and pulverized. The powdered form was completely immersed on 80% ethanol for 1 hr at room temperature .The suspension was filtered using #1 filter paper (Whatman Inc. Hillsboro, OR, USA.) twice. The filtrate was incubated at 40°C, until the ethanol was completely evaporated (Pineda, 2009).

Animals

Fifteen Sprague-Dawley rats weighing 90-120 grams (6 weeks old) purchased from BioPhilippines, Manila. The animals were housed in a standard cage in a room maintained at 24± 2°C with controlled lighting (lights on 8:30- 20:30). The animals had free access to dry pellet and water throughout the course of study. They were acclimatized for 2 weeks before the experimental procedures. The test group received 400 mg/kg of the *Ipomea batatas* crude leaf extract while the control group received Livatone® at the same dosage. NSS was used as a negative control. All the experimental procedures and protocols used in the study were reviewed and approved by the BAI.

Hepatotoxicity

The fifteen Sprague-Dawley rats was divided into three groups. Group 1 was treated with NSS for seven days and received acetaminophen suspension by oral route in a dose of 500 mg/kg/day and 12 oz of ethanol for three days. Group II, the control group received Livatone®for seven days and treated with acetaminophen and ethanol for three days. Group III, served as the test group and was treated with the crude extract of *Ipomeabatatas Linn.* For seven days and was administered with the same treatment of' acetaminophen and ethanol. After the treatment period, the rats' liver integrity was assessed by testing the ALT, AST and total bilirubin using spectrophotometric methods (EliTech, France*)* using a semi-automated spectrophotometer (StatFax, Awareness Technology, USA).

Statistical Analysis

Each result was expressed as means ± Standard Error. The grouped data was evaluated statistically using one-way analysis of variance (ANOVA) and t-test for independent variables. $p<0.05$ was considered significant (Zhou et al., 2009)

RESULTS

After the induction of liver damage using acetaminophen and alcohol, the activities of enzymes ALT, AST, and bilirubin showed increased levels. The Livatone® , at 400 mg/kg body weight showed close results to 400mg/kg body weight of *Ipomea batatas Linn.*flavonoids in terms of controlling the liver enzymes activity.

The post induction of 500mg/kg body weight of acetaminophen and 5ml/kg body weight of 18% ethanol using 3ml normal saline solution via oral gavage greatly showed increased values of ALT, AST, and bilirubin on the negative group (Group 1) from 61.3-67.52 U/L (AST), 26.3-41.52 U/L (ALT) and 0.003U/L – 0.39 U/L (Bilirubin) within 3 days of continuous intoxication of the liver prior to pretreatment of NSS for 7 days (3 ml).

The induced hepatotoxicity using the same dosage of acetaminophen and alcohol used in the first group was done to the positive group (Group 2) who were treated with 400mg/kg body weight of Livatone® for 7 days prior to the post induction. The results showed increased AST, ALT and bilirubin levels from (2.64-68.6 U/L, 2.42-34.52 U/L and 0.032-0.1376 U/L respectively) at the first day but lowered at the last day of induction resulting to 49.52 U/L (AST) and 28.64 U/L (ALT) but with slight increase on bilirubin level (0.2162 U/L).

The test group were treated with 400 mg/kg body weight of flavonoids from the crude extract of *Ipomea batatas Linn.*for 7 days using 3 ml NSS vehicle via oral route. After induction of acetaminophen

and alcohol in the same dosage, the enzyme levels were increased in the 1st day by 52.2 U/L (AST), 22.54 U/L (ALT) and 0.186 U/L (Bilirubin) compared to their baseline values done before the whole course of treatment (2.44 U/L, 2.28 U/L and 0.094 U/L respectively). Its hepatoprotective property was evident on the third day of toxicity induction when the serum assay resulted to 37.92 U/L (AST), 25.7 U/L (ALT) and 0.1308 U/L (Bilirubin).

Statistical analysis of results through multiple comparison of means showed that there is no significant difference on the effect of 400 mg/kg body weight of *Ipomea batatas Linn.*crude extract and 400 mg/kg body weight of Livatone® at <0.05 significance while there was significance between the treatment of the crude extract and NSS only (negative control).

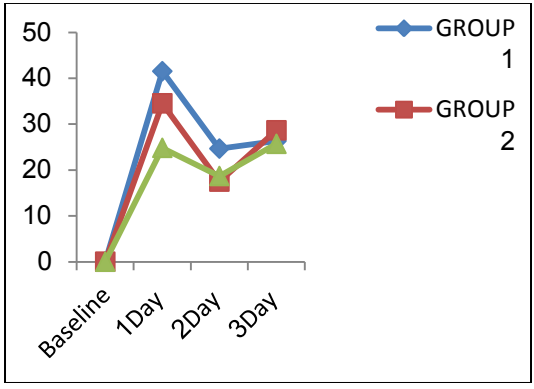

Figure 1. Effects of Acetaminophen and alcohol induction on rats treated with NSS, Livatone®, and flavonoids respectively, expressed on ALT from day 1 to day 3

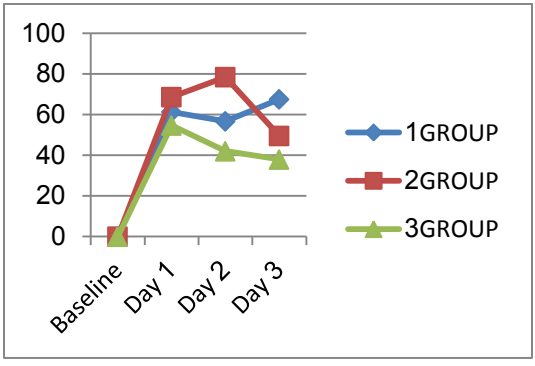

Figure 2. Effects of Acetaminophen and alcohol induction on rats treated with NSS, Livatone®, and flavonoids respectively, expressed on AST from day 1 to day 3

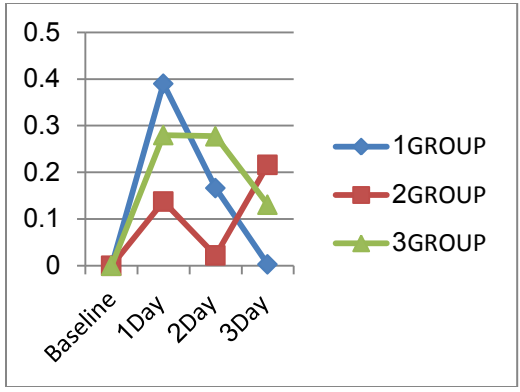

Figure 3. Effects of Acetaminophen and alcohol induction on rats treated with NSS, Livatone®, and flavonoids respectively, expressed on Bilirubin from day 1 to day 3

DISCUSSION AND CONCLUSIONS

Liver diseases are the most serious ailment and are mainly caused by toxic chemicals (excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil, etc). (Maheswari et al., 2008). Plant medicines have been used today to treat different liver diseases.

Studies proved that *Ipomoea batatas Linn.* has a high flavonoid content (Yan-Hwa et al., 2000) and this study is anchored on the theory that the flavonoids from its leaf crude extract will manifest a hepatoprotective effect on the liver in comparison to a commercially-available liver tonic at the same dosage.

This study establishes the effect of the crude extract of *Ipomoea batatas Linn.*as an alternative for Livatone® which is generally accepted as a liver tonic. This study also reveals that both Livatone® and the crude leaf extract of the said plant have the same potency and therapeutic efficacy in protecting the liver of both the Sprague-Dawley rats in the control group and in the test group at a dose of 400 mg/kg body weight. It is therefore concluded that the crude leaf extract of *Ipomea batatas Linn.* has the potential as an alternative liver tonic with hepatoprotective properties. Since the study utilized a crude extract from the plant, it cannot be fully elucidated if it is the flavonoid content of the crude extract that exerted the hepatoprotective effect. Such determinations can be done through the use of purification techniques to isolate the flavonoid content of the extract.

RECOMMENDATIONS

Future studies on the acute lethal dose of *Ipomea batatas Linn.* crude extract is highly recommended. It is also recommended that the isolation of the primary and secondary metabolites of the plant particularly its flavonoid content and other bioactive components be performed and tested for hepatoprotective effects using a larger population of animal and human models.

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